IFWO

RAW SEQUENCE LISTING   DATE: 08/26/2004
PATENT APPLICATION: US/10/766,993   TIME: 11:37:09

Input Set : A:\-8-1.app
Output Set: N:\CRF4\08262004\J766993.raw

```
 3 <110> APPLICANT: Chang, Chia-Hwa
 4        Liu, Xiaowen
 5        Lewicki, John A.
 6        Xu, Qiang
 7        Osel, Inc.
 9 <120> TITLE OF INVENTION: Surface Expression of Biologically Active Proteins in
10        Bacteria
12 <130> FILE REFERENCE: 016976-000810US
14 <140> CURRENT APPLICATION NUMBER: US 10/766,993
15 <141> CURRENT FILING DATE: 2004-01-28
17 <150> PRIOR APPLICATION NUMBER: US 60/443,619
18 <151> PRIOR FILING DATE: 2003-01-29
20 <160> NUMBER OF SEQ ID NOS: 89
22 <170> SOFTWARE: PatentIn Ver. 2.1
24 <210> SEQ ID NO: 1
25 <211> LENGTH: 1765
26 <212> TYPE: PRT
27 <213> ORGANISM: Lactobacillus jensenii 1153
29 <220> FEATURE:
30 <223> OTHER INFORMATION: genomic C14 cell wall anchor sequence
32 <220> FEATURE:
33 <221> NAME/KEY: DOMAIN
34 <222> LOCATION: (1528)..(1765)
35 <223> OTHER INFORMATION: CWA200 cell wall associated region with anchor
36        motif
38 <220> FEATURE:
39 <221> NAME/KEY: PEPTIDE
40 <222> LOCATION: (1730)..(1734)
41 <223> OTHER INFORMATION: anchor motif, sorting signal, cell wall targeting
42        region
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46  1               5                  10                  15
48 Ile Thr Gly Lys Val Asn Gly Leu Thr Thr Ser Gly Ile Ser Asp Ile
49              20                  25                  30
51 Asn Lys His Phe Leu Tyr Leu Gln Ser Glu Gly Ser Ala Arg Asp Leu
52           35                  40                  45
54 Thr Ile Asn Gly Asn Gly His Arg Ile Asn Phe Ala Gly Tyr Ser Ile
55        50                  55                  60
57 Ala Leu Gln Asn Lys Asn Tyr Thr Asn Ala Ala Asn Pro Trp Asn Ile
58  65                  70                  75                  80
60 Thr Leu Lys Asp Met Thr Ile Glu Gly Ser Lys Tyr Asp Tyr Ser Pro
61              85                  90                  95
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ENTERED

(ps.6)

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63 Ile Ser Phe Tyr Gly Arg Lys Ser Asn Thr Glu Asn Ser Lys Leu Thr
64          100                 105                 110
66 Phe Asp Gly Val Thr Ala Asn Leu Asn Asp Arg Pro Leu Val Asp Lys
67          115                 120                 125
69 Tyr Gly Glu Asn Leu Pro Val His Phe Ala Gly Glu Asn Asn Ile Thr
70     130                 135                 140
72 Leu Asn Asn Met Ser Ile Gly Tyr Asn Leu Val Thr Gly Lys Thr Val
73 145                 150                 155                 160
75 Lys Phe Asp Ser Gly Asn Thr Thr Phe Asn Val Asp Gly Lys Val Thr
76              165                 170                 175
78 Gly Asn Ser Ile Asn Pro Asp Asn Trp Val Ile Arg Ser Thr Glu Asn
79              180                 185                 190
81 Ala Ser Asn Ser Glu Asn Pro Ser Thr Leu Ile Asn Glu Gly Ala Thr
82          195                 200                 205
84 Val Thr Ile Asn Ala Lys Ser Asp Asp Leu Arg Gly Ile Tyr Ala Gly
85     210                 215                 220
87 Arg Gln Leu Thr Ala Gly Gln Pro Ile Tyr Gly Val Thr Val Ile Asn
88 225                 230                 235                 240
90 Gly Thr Leu Asn Ala Lys Met Ala Ala Gly His Ser Thr Ala Ile Trp
91              245                 250                 255
93 Ser His Asp Leu Glu Ile Gly Lys Lys Gly Asn Val Thr Ile His Thr
94              260                 265                 270
96 Lys Gln Thr Asn Gln Ala Asp Gly Val Glu Asn Gly Thr Ser Asn Ser
97          275                 280                 285
99 Val Thr Asn Tyr Asn Gly Thr His Tyr Ala Pro Ile Ser Leu Gly Val
100    290                 295                 300
102 Gly Pro Ile Ser Ser Val Ala Ser Pro Leu Ser Lys Gln Thr Val Ser
103 305                 310                 315                 320
105 Leu Ile Asn Asn Gly Ser Leu Thr Ile Ile Arg Asp Thr Ala Lys Lys
106              325                 330                 335
108 Thr Leu Val Pro Leu Ile Ser Met Gly Asp Gly Ser Leu Ser Ser Asn
109              340                 345                 350
111 Thr Thr Leu Lys Phe Ser Val Gly Ala Gly Ala Thr Leu Asp Leu Gln
112          355                 360                 365
114 Asp Lys Ala Gly Thr Phe Arg Tyr Gly Ile Glu Pro Ser Thr Pro Leu
115     370                 375                 380
117 Asn Gly Leu Val Thr Leu Trp Gly Thr Ser Gly Thr Asp Leu Leu Glu
118 385                 390                 395                 400
120 Phe Leu Thr Pro Ala Tyr Val Asn Leu Gln Arg Thr Gly Asp Ile Arg
121              405                 410                 415
123 Gly Thr Leu Ile Arg Met Glu Gly Val Tyr Asn Ser Thr Thr Val Asn
124              420                 425                 430
126 Gly Pro Thr Pro Val Ala Gln Trp Asp Gln Gly Asn Lys Thr Thr Thr
127          435                 440                 445
129 Pro Asn Asp Val Trp Tyr Val Arg Tyr Leu Ile Ser Ala Asn Gln Trp
130     450                 455                 460
132 Gly Asn Asn Ser Gly Gln Phe Met Gly Lys Asp Gln His Pro Asn Thr
133 465                 470                 475                 480
135 Val Val Ala Lys Lys Gly Val Asp Thr Leu Tyr Asn Ser Asn Ala Thr
```

```
136                   485                  490                  495
138 Val Leu Met Ser Lys Asn Gln Gly Ala Asp Lys Tyr Glu Asn Gly Thr
139                   500                  505                  510
141 Met Pro Thr Glu Val Gln Gln Ala Leu His Leu Asn Ser Phe Leu Asn
142           515                  520                  525
144 Asn Phe Asn Phe Trp Arg Pro Gln Arg Met Ala Met Gly Ser Lys Leu
145       530                  535                  540
147 Asn Asp Asn Pro Asp Val Lys Ile Asp Asp Phe Asp Lys Tyr His Ala
148 545                   550                  555                  560
150 Glu Ala Gln Thr Ile Asp Gly Thr Thr Arg Gln Thr Leu Ser Asp Leu
151               565                  570                  575
153 Asp Ala Asn Lys Gly Leu Lys Asp Leu Ile Gly Pro Asp Glu Gln Pro
154           580                  585                  590
156 Ile Thr Asp Phe Lys Asp Ile Val Lys His Val Thr Trp Tyr Asn Ser
157           595                  600                  605
159 Ala Thr Asp Lys Asp Glu Trp Asn Lys Ile Met Ile Gln Pro Thr Asp
160       610                  615                  620
162 Ser Lys Asp Pro Ser Ala Arg Val Pro Tyr Pro Glu Pro Gln Asn Pro
163 625                   630                  635                  640
165 Thr Gly Asn Leu Lys Thr Thr Asp Gly Phe Ala Trp Ala Lys Val Thr
166               645                  650                  655
168 Tyr Ala Asp Gly Ser Val Asp Phe Val Lys Ile Pro Leu Lys Val Thr
169           660                  665                  670
171 Glu Lys Lys Tyr Ser Glu Glu Leu Thr Pro Ser Tyr Pro Gly Val Ser
172           675                  680                  685
174 Val Glu Gln Gly Lys Ser Asp Ser Val Asp Pro Ser Phe Lys Asp Glu
175       690                  695                  700
177 Asn Asp Lys Ala Ala Asp Ala Pro Ala Gly Thr Lys Tyr Thr Ala Gly
178 705                   710                  715                  720
180 Glu Asn Thr Pro Asp Trp Ile Lys Val Asp Pro Asp Thr Gly Lys Val
181               725                  730                  735
183 Thr Val Ser Pro Thr Asp Asp Thr Ser Val Gly Ser His Asp Ile Ser
184           740                  745                  750
186 Val Thr Val Thr Tyr Pro Asp Ser Ser Thr Asp Gln Leu Thr Val Pro
187           755                  760                  765
189 Val Thr Val Thr Glu Lys Ser Asn Leu Ala Glu Lys Tyr Pro Val Ser
190       770                  775                  780
192 Tyr Asp Lys Leu Asn Val Glu Lys Pro Ser Gly Asp Thr Pro Ala Thr
193 785                   790                  795                  800
195 Gly Ala Val Asp Pro Lys Ala Ala Ala Asp Met Pro Glu Gly Ala Ile
196               805                  810                  815
198 Thr Gly Tyr Glu Lys Gly Asp Phe Asp Ala Pro Ala Gly Val Thr Ile
199           820                  825                  830
201 Asp Val Asn His Asp Thr Gly Lys Val Thr Ala Ser Val Gly Lys Asn
202           835                  840                  845
204 Ala Thr Leu Gly Ser Phe Glu Val Pro Val Lys Val Thr Tyr Ser Asp
205       850                  855                  860
207 Gly Thr Tyr Ala Glu Val Lys Val Pro Val Ser Ile Thr Gly Asn Lys
208 865                   870                  875                  880
```

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```
210 Val Asp Pro Gly Ser Gly Asp Val Val Tyr Tyr Gly Asp Gln Ser Met
211              885             890             895
213 Val Val Phe Asn Gly Asn Leu Thr Thr Val His Lys Thr Thr Asp Ser
214              900             905             910
216 His Glu Leu Ser Ala Lys Asp Ser Ala Phe Gln Thr Ile Thr Tyr Tyr
217          915             920             925
219 Ser Asp Trp Asn Lys Lys Gly Asn Ile Val Ser Asp Tyr Asn Lys His
220          930             935             940
222 Val Ile Tyr Lys Leu Ser Ala Asp Gly Thr Lys Tyr Val Asn Glu Ala
223 945             950             955             960
225 Asp Ala Thr Asp Ser Phe Asp Ala Ser Ala Ile Ser Phe Asn Trp Gln
226              965             970             975
228 Lys Gly Tyr Glu Val Asn Thr Gly Val Asp Asn Phe Ser Asn Gly Ser
229              980             985             990
231 Ala Asp Thr Leu Tyr Gln Leu Glu Lys Gly Ala Val Asn Ser Glu Glu
232          995             1000            1005
234 Gln Thr Asp Ala Asn Asp Pro Ser Gly Leu Ala Gly Asn Ser Lys Tyr
235     1010            1015            1020
237 Arg Tyr Asp Phe Ser Ile Ser Asp Thr Asn Val Leu Gln Lys Leu Gly
238 1025            1030            1035            1040
240 Leu Ser Pro Ala Gly Tyr Asn Ala Trp Ala Asn Val Tyr Tyr Asn Phe
241              1045            1050            1055
243 Leu Gly Ala Thr Gly Lys Ile Asn Ile Pro Val Asn Tyr Gly Ser Glu
244              1060            1065            1070
246 Val Ser Thr Asp Glu Ala Gly Ile Lys Asn Tyr Leu Ala Thr Asn Ser
247     1075            1080            1085
249 Ile Ser Gly Lys Thr Phe Val Asn Gly Asn Pro Thr Gly Ile Lys Trp
250   1090            1095            1100
252 Ala Glu Asn Gly Met Pro Gly Lys Asp Gly Lys Phe Ala Ala Ser Asn
253 1105            1110            1115            1120
255 Met Thr Gly Ile Val Glu Phe Thr Phe Asp Asn Gly Thr Lys Leu Asn
256              1125            1130            1135
258 Val Gln Val Thr Phe Lys Thr Gly Ser His Val Ser Thr Ser Gly Ser
259          1140            1145            1150
261 Lys Val Asn Asp Asp Thr Asn Leu Tyr Val Glu Arg Thr Ile Glu Tyr
262          1155            1160            1165
264 Asp Val Thr Gly Thr Gly His Ser Pro Ile Asn Ser Val Thr Gln Lys
265     1170            1175            1180
267 Val His Tyr Val Arg Asp Gly Tyr His Lys Ile Asn Ala Asp Gly Thr
268 1185            1190            1195            1200
270 Asp Ala Gly Glu Ile Ile Trp Asn Glu Trp Lys Leu Ala Asp Gly Gln
271              1205            1210            1215
273 Thr Ala Glu Phe Pro Glu Tyr Ser Val Asp Gln Ile Thr Gly Tyr Asp
274              1220            1225            1230
276 Ala Tyr Ile Asn Gly Ala Lys Ala Thr Gln Val Asp Ala Ala Lys Val
277          1235            1240            1245
279 Ala Glu Thr Asn Gly Thr Pro Gln Asn Gly Gln Asn Ile Thr Val Thr
280     1250            1255            1260
282 Tyr Lys Lys Gln Asn Ser Thr Pro Val Pro Tyr Lys Pro Gly Lys Asp
```

```
283 1265                1270                1275                1280
285 Gly Val Asn Asp Ala Ile Asn Arg Tyr Val Thr Arg Thr Ile Ile Val
286                1285                1290                1295
288 Lys Glu Pro Gly Lys Glu Pro Gln Thr Ile Thr Gln Thr Val His Phe
289            1300                1305                1310
291 Thr Asn Glu Asp Lys Asp Gly Asn Ser Gly Tyr Lys Asp Pro Val Thr
292        1315                1320                1325
294 Gly Glu Ile Lys Tyr Asn Thr Asp Trp His Val Ala Ser Asp Leu Asn
295    1330            1335                1340
297 Ala Lys Thr Gly Ser Trp Glu Glu Tyr Thr Ala Pro Ser Val Thr Gly
298 1345            1350                1355                1360
300 Tyr Thr Pro Ser Gln Ala Lys Val Glu Ala Lys Thr Val Thr Ala Glu
301            1365                1370                1375
303 Thr Glu Ala Ala Ser Val Thr Ile Ser Tyr Thr Lys Asn Ala Asp Ile
304            1380                1385                1390
306 Pro Val Pro Tyr Lys Pro Gly Lys Asp Gly Val Asn Asp Ala Ile Asn
307        1395                1400                1405
309 Arg Tyr Val Thr Arg Thr Ile Ile Val Lys Glu Pro Gly Lys Glu Pro
310    1410            1415                1420
312 Gln Thr Ile Thr Gln Thr Val His Phe Thr Asn Glu Asp Lys Asp Gly
313 1425            1430                1435                1440
315 Asn Ser Gly Tyr Lys Asp Pro Val Thr Gly Glu Ile Lys Tyr Asn Thr
316            1445                1450                1455
318 Asp Trp His Val Ala Ser Asp Leu Asn Ala Lys Thr Gly Ser Trp Glu
319            1460                1465                1470
321 Glu Tyr Thr Ala Pro Ser Val Thr Gly Tyr Thr Pro Ser Gln Ala Lys
322        1475                1480                1485
324 Val Glu Ala Lys Thr Val Thr Ala Glu Thr Glu Ala Ala Ser Val Thr
325    1490            1495                1500
327 Ile Ser Tyr Thr Lys Asn Ala Asp Ile Pro Val Pro Phe Asp Pro Ser
328 1505            1510                1515                1520
330 Asn Lys Asp Met Tyr Arg Glu Val Thr Arg Thr Ile Asn Val Val Asp
331            1525                1530                1535
333 Pro Ile Thr Gly Lys Ile Ser Thr Ser Val Gln Thr Ala Lys Phe Thr
334            1540                1545                1550
336 Arg Glu Asp Lys Asn Ser Asn Ala Gly Tyr Thr Asp Pro Val Thr Gly
337        1555                1560                1565
339 Lys Thr Thr Met Asn Pro Trp Thr Pro Ala Lys Gln Gly Leu Arg Ala
340    1570            1575                1580
342 Val Asn Val Glu Gln Ile Lys Gly Tyr Val Ala Lys Val Asp Gly Asn
343 1585            1590                1595                1600
345 Val Asp Ala Val Val Val Thr Pro Asp Ser Ala Asn Met Val Val Thr
346            1605                1610                1615
348 Ile Thr Tyr Gln Ala Asn Lys Pro Glu Gly Gln Asn Ile Thr Val Lys
349        1620                1625                1630
351 Lys Asp Thr Val Pro Asp Pro Ala Asp Gly Ile Lys Asn Lys Asp Asp
352    1635            1640                1645
354 Leu Pro Asp Gly Thr Lys Tyr Thr Trp Lys Glu Val Pro Asp Val Asn
355    1650            1655                1660
```

RAW SEQUENCE LISTING ERROR SUMMARY   DATE: 08/26/2004
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Input Set : **A:\-8-1.app**
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Please Note:
Use of n and/or Xaa have been detected in the Sequence Listing. Please review the Sequence Listing to ensure that a corresponding explanation is presented in the <220> to <223> fields of each sequence which presents at least one n or Xaa.

Seq#:9; Xaa Pos. 3
Seq#:29; Xaa Pos. 3
Seq#:30; Xaa Pos. 3
Seq#:31; Xaa Pos. 3,6

VERIFICATION SUMMARY   DATE: 08/26/2004
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Input Set : **A:\-8-1.app**
Output Set: **N:\CRF4\08262004\J766993.raw**

```
L:1221 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:9 after pos.:0
L:1527 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:29 after pos.:0
L:1546 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:30 after pos.:0
L:1566 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:31 after pos.:0
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